UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF *IN VITRO* PROPAGATION TECHNIQUES FOR *ENDOSPERMUM MALACCENSE* M.A AND *SHOREA PARVIFOLIA* DYER

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FH 2003 13

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By

AZIAH MOHD YUSOFF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

April 2003

Abstract of thesis submitted to the Senate of University Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

DEVELOPMENT OF *IN VITRO* PROPAGATION TECHNIQUES FOR *ENDOSPERMUM MALACCENSE* M.A AND *SHOREA PARVIFOLIA* DYER

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Chairman: **Associate Prof. Dr. Nor Aini Ab. Shukor**

Faculty: **Forestry**

Endospermum malaccense M.A and *Shorea parvifolia* Dyer are two commercially important timber species identified as potential plantation species. The procurement and storage of their seeds are difficult and is a major hindrance to plantation establishment. Development of micropropagation techniques is being pursued to provide an alternative in planting stock production.

Micropropagation of *E. malaccense* was achieved through *in vitro* production of plants through axillary shoots development from nodal segment explants of an elite tree. The explants were initially washed in 10 changes of sterile distilled water, followed by 5 changes of 0.05% (v/v) Tween 20 solution for 10 minutes each. This was then followed by rinsing in 10 changes of sterile distilled water and subsequently sterilised in a solution comprising 60 % (v/v) Clorox and 0.05% v/v Tween 20 for 10 minutes. After

which they were rinsed 10 times in 300ml sterile distilled water and finally immersed in 70% (v/v) ethanol for 1 minute. Shoots were induced on the nodal segment explants in MS basal salts supplemented with 22.2 x 10^{-6} M or 44.4 x 10^{-6} M. For shoot multiplication, the best medium is MS supplemented with 44.4 x 10^{-6} M BAP and solidified with a mixture of 1.7 g Gelrite and 4g Bacto agar per liter. *In vivo* rooting with Seradix 3 was more suitable for *in vitro* produced shoots of *E. malaccense*, compared with an *in vitro* rooting technique.

For *S. parvifolia*, high contamination was observed in all explant types. Multiple shoots were induced on nodal segments culture in WPM solid medium supplemented with 10^{-5} M BAP or 10^{-6} TDZ but were non-amenable to further subculture. Callus developed from immature seeds with gelatinous endosperm termed as embryonic masses in WPM supplemented with 10^{-4}M CPA induced callus formation. Globular shaped callus developed upon subculture. Histological examination of the globular shaped callus showed no evidence of somatic embryos formation. The globular structure was similar to the development of nodules.

The contaminants found on the immature seeds included a fungus, *Collectotrycum* spp. and a range of bacteria which are as follows: -*Kleibsella planticola*, *Enterobacter agglomerans*, *Erwina* spp. (*E. uredora* or *E. herbicola*), *Serratia odorifera*, *Serratia marcesens*, *Serratia proteomaculans*, *Morganella moragnii*, *Kluyera ascorbata*. A fern, *Asplenium nidus* was found contaminating the nodal segment cultures.

Abstrak tesis yang dikemukakan kepada Senat Universityi Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

PEMBENTUKAN TEKNIK MIKRO PERAMBATAN UNTUK *ENDOSPERMUM MALACCENSE* M.A DAN *SHOREA PARVIFOLIA* DYER

Oleh

AZIAH BINTI MOHD YUSOFF

April 2003

Pengerusi: **Prof. Madya Dr. Nor Aini Ab. Shukor**

Fakulti: **Perhutanan**

Endospermum malaccense M.A dan S*horea parvifolia* Dyer, merupakan dua sepsis balak yang berpotensi ditanam secara ladang. Masalah pengutipan dan penyimpanan bijibenih menghindar kemajuan peladangan hutan secara komersial. Pembentukan teknik mikroperambatan dapat mengatasi masalah pembekalan bahan tanaman.

Mikroperambatan *E. Malaccense* tercapai apabila pembentukan pucuk aksil terbentuk ke atas eksplan bahagian nod pokok elit. Eksplan dibasmikuman dengan mencuci 10 kali dengan air suling steril, diikuti dengan 5 pencucian dalam larutan 0.05% (isipadu/isipadu) Tween 20 selama 10 minit, diikuti pula dengan 10 pertukaran air suling steril. Kemudian dicuci pula dalam larutan 60% (isipadu/isipadu) Clorox

dicampur dengan 0.05% (isipadu/isipadu) Tween 20 selama 10 minit. Kemudian dicuci dengan 10 kali dengan 300ml air suling steril dan akhir sekali direndam didalam 70% (isipadu/isipadu) etanol. Media MS dicampur dengan 22.2 x 10^{-6} M or 44.4 x 10^{-6} M sesuai dalam pengaruhan pembentukan pucuk. Gandaan pucuk tercapai dalam campuran medium MS dan 44.4 x 10^{-6} M BAP yang dipepejalkan dengan jel campuran 1.7 g Gelrite and 4g Bacto agar se liter. Teknik pengakaran *in vivo* dengan Seradix 3 lebih sesuai dibandingkan dengan teknik *in vitro*.

Untuk *S. parvifolia*, kadar kontaminasi tinggi dihadapi dalam kultur semua jenis eksplan. Pembentukan pucuk berbilang tercapai apabila bahagian nod di kulturkan didalam medium WPM yang ditambah dengan 10^{-5} M BAP atau 10^{-6} M TDZ. Pertambahan pucuk tidak tercapai dalam semua jenis medium. Pembentukan kalus terjadi ke atas eksplan endoperma cair di dalam medium WPM ditambah dengan 10^{-4} M CPA. Pengsubkulturan menghasilkan kalus berbentul globul. Kajian histology menunjukkan bahawa struktur ini adalah nodul.

Bijibenih muda dikontaminasikan oleh sejenis kulat, *Collectotrycum* spp. Dan pelbagai jenis bacteria seperti berikut:--*Kleibsella planticola, Enterobacter agglomerans, Erwina spp. (E. uredora or E. herbicola), Serratia odorifera, Serratia marcesens, Serratia proteomaculans, Morganella moragnii, Kluyera ascorbata*. Sejenis paku penumpang *Asplenium nidus* juga didapati menkontaminasikan kultur.

ACKNOWLEDGEMENTS

In the name of Allah S.W.T. The Most Gracious. The Most Merciful.

First and foremost I would like to convey my appreciation to my present employer the Golden Hope Research Sdn. Bhd. (GHRSB), for allowing me to continue my studies till completion. My appreciation goes to the Executive Director of Golden Hope Research Sdn. Bhd., Dr. Mohd Hashim Tajudin, for his support and to Dr. Mohd Nor Ghani, the Research Controller of the BioScience Division for his continuous encouragement. I am indebted to the management of the Forest Research Institute of Malaysia (FRIM), my former employer, for the permission to pursue my studies during my tenure in FRIM. Special thanks goes to the Director General Dato' Abd. Razak bin Mohd Ali, who has been very supportive of my pursuit to complete my studies.

I deeply appreciate the support of my supervisor Assoc. Prof. Dr. Nor Aini Shukor for her patience, encouragement and guidance. My heartfelt thanks go to the members supervisory committee, Assoc. Prof. Dr. Kamis Awang and Assoc. Prof. Dr. Midhzar Abdul Kadir for their suggestions and comments leading to the completion of this thesis.

To my former Research Assistant in FRIM, Pn. Sharifah Maulana, I thank her for assisting me in my experiments as well as her patience and fruitful suggestions. To my

present assistant at GHRSB, Pn. Ruziah Ahmad, I thank her for her assistance in the final layout and final touches of my thesis and to Asmida of FRIM, I am grateful for her assistance in the preparation of the colour prints for this thesis.

A million thanks goes to the members of the tissue culture laboratory at FRIM, Pn Fadhilah, Pn. Haliza, Pn. NorAsmah, Pn Halilah, Pn Normah, Pn Rozidah, Cik Sabariah, Pn Naemah, Pn Rukiah and Nizam for their cooperation.

To my former colleagues at FRIM, Dr. Ab. Rasip, Mohd Nor, David McKellar, Norhara, Dr. Ilham and numerous others, I thank you all for your support and suggestions.

To my beloved family, Mansor, my husband and children, Murni, Maizura, Marlina and Muhd. Isa, and last but not least to my mother, Cik An, my deepest appreciation, for their continuous encouragement, understanding and most of all, for their sacrifices in bearing all the shortcomings during these trying period, in order for me to complete this thesis. Thank you.

I certify that an Examination Committee met on 16th April 2003 to conduct the final examination of Aziah Mohd Yusoff on her Doctor of Philosophy thesis entitled "Development on *In Vitro* Propagation Techniques for *Endospermum Malaccense* M.A. and *Shorea Parvifolia* Dyer" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

(AZIAH MOHD YUSOFF)

Date : 21st MAY 2003

TABLE OF CONTENTS

LISTS OF TABLES

LIST OF FIGURES